Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 2-16, 18-29, and 61-79 are pending in the application, with claim 2 being the independent claim. Claims 1, 17, and 30-60 have been cancelled without prejudice to or disclaimer of the subject matter therein. Applicants reserve the right to pursue the canceled subject matter in a co-pending application. Claims 8, 9, 16, and 63 have been amended and claims 68-79 have been added. Support for amended claims 8, 9, and new claims 68-72 can be found *inter alia*, on page 43, line 26 to page 44, line 5. Support for amended claim 16, and new claims 73-79 can be found *inter alia*, on page 28, lines 4-17.

This Amendment is being filed along with a request for continued examination. Therefore, entry of the new and amended claims is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and further request that they be withdrawn.

Objection to the Title

Applicants have amended the title as suggested by the Examiner. Applicants believe that the title is now clear and respectfully request reconsideration and removal of the objection.

Objection to the Abstract

Applicants have amended the abstract to include a description of the novel *ask* mutant as requested by the Examiner. Applicants believe that the abstract is now complete and respectfully request reconsideration and removal of the objection.

Rejections Under 35 U.S.C. § 112, first paragraph

The rejection of claims 8, 9, 16, 25, and 26 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed subject matter was maintained. Applicants respectfully traverse.

Not in acquiescence to the Examiner's rejection, but rather to advance prosecution, Applicants have amended claims 8 and 16 to recite the host cell of claim 6 expressing one of the enzymatic activities of the lysine biosynthesis pathway, and to include specific sequence identifiers which identify the captioned gene, respectively. Support for these amendments can be found, *inter alia*, on page 43, line 26 to page 44, line 5 and Figure 1; and page 28 lines 4-17.

Claim 63 is rejected under 35 U.S.C. § 112, first paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Not in acquiescence to the Examiner's rejection, but rather to advance prosecution, Applicants have amended claim 63 to clarify the meaning of the claim. Therefore, Applicants respectfully request reconsideration and removal of the rejection.

Rejections Under 35 U.S.C. § 112, second paragraph

The rejection of claims 8, 9, 16, 25, and 26 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention was maintained. Applicants respectfully traverse.

Not in acquiescence to the Examiner's rejection, but rather to advance prosecution, Applicants have amended claim 8 to remove the terms '*lysA* and *ORF2* and rather recite the enzymatic characteristics of the host cells. Furthermore, Applicants have amended claim 16 to include specific sequence identifiers which identify the captioned gene. Therefore, Applicants respectfully request reconsideration and removal of the rejection.

Other Matters

The Examiner indicated on the Office Action Summary under Disposition of Claims, and again on page 8 of the Detailed Action, that claims 2-7, 10-15, 17-24, 27-29, 61, 62, and 64-69 are allowed. Applicants would like to draw the Examiner's attention to the fact that at that time, claims 68 and 69 had not been submitted and were not pending. Therefore, Applicants respectfully request that for clarity the Examiner acknowledge that claims 2-7, 10-15, 17-24, 27-29, 61, 62, and 64-**67** were allowed in the Office Action of Paper 18.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

In the Title:

The following Title of the Invention was substituted for the pending Title of the Invention:

Polynucleotides Encoding a Feedback Resistant Aspartokinase from *Corynebacterium.*

[Increased Lysine Production by Gene Amplification Using Coryneform Bacteria.]

In the Claims:

Claims 1, 17, and 30-60 were cancelled.

The following claim 8 was substituted for the pending claim 8:

8. (Twice amended) The method of claim 6 wherein said host cell expresses one of the following:

(a) aspartate-semialdehyde dehydrogenase activity;

(b) dihydrodipicolinate synthase activity;

(c) dihydrodipicolinate reductase activity;

(d) diaminopimelate dehydrogenase activity; and

(e) diaminopimelate decarboxylase activity.

[8. The method of claim 6 wherein said polynucleotide molecule further comprises at least one of the following:

(a) a nucleic acid molecule encoding a *Corynebacterium* species lysine pathway *asd* amino acid sequence;

(b) a nucleic acid molecule encoding a *Corynebacterium* species lysine pathway *dapA* amino acid sequence;

(c) a nucleic acid molecule encoding a *Corynebacterium* species lysine pathway *dapB* amino acid sequence;

(d) a nucleic acid molecule encoding a *Corynebacterium* species lysine pathway *ddh* amino acid sequence;

(e) a nucleic acid molecule encoding a *Corynebacterium* species lysine pathway *'lysA* amino acid sequence;

(f) a nucleic acid molecule encoding a *Corynebacterium* species lysine pathway *lysA* amino acid sequence; and

(g) a nucleic acid molecule encoding a *Corynebacterium* species lysine pathway *ORF2* amino acid sequence.]

The following claim 9 was substituted for the pending claim 9:

9. (Twice amended) The method of claim 8 further comprising screening for said <u>activity</u> [transformed polynucleotide molecule].

The following claim 16 was substituted for the pending claim 16:

16. (Once amended) An isolated polynucleotide molecule comprising:

(a) the polynucleotide molecule of claim 2; and

(b) at least one additional *Corynebacterium* species lysine pathway gene selected from the group consisting of:

(i) a nucleic acid molecule encoding [an] the *asd* polypeptide of SEQ ID NO:4;

(ii) a nucleic acid molecule encoding [an] the *dapA* polypeptide of SEQ ID NO:6;

(iii) a nucleic acid molecule encoding [an] the *dapB* polypeptide of SEQ ID NO:8;

(iv) a nucleic acid molecule encoding [an] the *ddh* polypeptide of SEQ ID NO:10;

(v) a nucleic acid molecule encoding [an] the *'lysA* polypeptide of SEQ ID NO:21;

(vi) a nucleic acid molecule encoding [an] the *lysA* polypeptide of SEQ ID NO:14; and

(vii) a nucleic acid molecule encoding [an] the *ORF2* polypeptide of SEQ ID NO:16.

The following claim 63 was substituted for the pending claim 63:

63. (Twice amended) The isolated polynucleotide molecule of claim 61 wherein said promoter is operably [directly] linked to the nucleotide sequence encoding SEQ ID NO:2 [encoded polypeptide sequence].

New claims 68-79 were added.

68. The method of claim 8 wherein said activity is aspartate-semialdehyde dehydrogenase activity.

69. The method of claim 8 wherein said activity is dihydrodipicolinate synthase activity.

70. The method of claim 8 wherein said activity is dihydrodipicolinate reductase activity.

71. The method of claim 8 wherein said activity is diaminopimelate dehydrogenase activity.

72. The method of claim 8 wherein said activity is diaminopimelate decarboxylase activity.

73. The isolated polynucleotide molecule of claim 16, wherein said additional *Corynebacterium* species lysine pathway gene is the *asd* polypeptide of SEQ ID NO:4.

74. The isolated polynucleotide molecule of claim 16, wherein said additional *Corynebacterium* species lysine pathway gene is the *dapA* polypeptide of SEQ ID NO:6.

75. The isolated polynucleotide molecule of claim 16, wherein said additional *Corynebacterium* species lysine pathway gene is the *dapB* polypeptide of SEQ ID NO:8.

76. The isolated polynucleotide molecule of claim 16, wherein said additional *Corynebacterium* species lysine pathway gene is the *ddh* polypeptide of SEQ ID NO:10.

77. The isolated polynucleotide molecule of claim 16, wherein said additional *Corynebacterium* species lysine pathway gene is the *'lysA* polypeptide of SEQ ID NO:21.

78. The isolated polynucleotide molecule of claim 16, wherein said additional *Corynebacterium* species lysine pathway gene is the *lysA* polypeptide of SEQ ID NO:14.

79. The isolated polynucleotide molecule of claim 16, wherein said additional *Corynebacterium* species lysine pathway gene is the *ORF2* polypeptide of SEQ ID NO:16.

In the Abstract:

The following abstract was substituted for the pending abstract:

The invention provides methods to increase the production of an amino acid from *Corynebacterium* species by way of the amplification of amino acid biosynthetic pathway genes in a host cell chromosome. In a preferred embodiment, the invention provides methods to increase the production of L-lysine in *Corynebacterium glutamicum* by way of the amplification of L-lysine biosynthetic pathway genes in a host cell chromosome. The invention also provides novel processes for the production of an amino acid by way of the amplification of amino acid biosynthetic pathway genes in a host cell chromosome and/or by increasing promoter strength. In a preferred embodiment, the invention provides processes to increase the production of L-lysine in *Corynebacterium glutamicum* by way of the amplification of L-lysine biosynthetic pathway genes in a host cell chromosome. The invention also provides novel isolated nucleic acid molecules for L-lysine biosynthetic pathway genes of *Corynebacterium glutamicum* such as a naturally occurring, feedback- sensitive form of aspartokinase (*ask*) resulting from a threonine to isoleucine mutation at amino acid residue 380 in the *ask* gene of ATCC 21529 [threonine-mutated, feedback-sensitive aspartokinase (*ask*)], aspartate-semialdehyde dehydrogenase (*asd*), dihydrodipicolinate synthase (*dapA*), dihydrodipicolinate reductase

(*dapB*), diaminopimelate dehydrogenase (*ddh*), and diaminopimelate decarboxylase (*lysA*).